FEBS Letters 586 (2012) 122–126

journal homepage: www.FEBSLetters.org

Interaction of protein inhibitor of activated STAT 2 (PIAS2) with receptor of activated C kinase 1, RACK1

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ARTICLE INFO

Article history:
Received 21 October 2011
Revised 30 November 2011
Accepted 7 December 2011
Available online 26 December 2011

Edited by Gianni Cesareni

Keywords:
Yeast two-hybrid system
PIAS2
RACK1
Protein interacting

ABSTRACT

In this study, the evolutionarily conserved intracellular adaptor protein, receptor of activated C kinase 1 (RACK1) was identified as a novel interaction partner of protein inhibitor of activated STAT 2 (PIAS2) using a yeast two-hybrid screening system. The direct interaction and co-localization of RACK1 with PIAS2 was confirmed by immunoprecipitation and immunofluorescence staining analysis, respectively. The 5th to 7th Trp-Asp 40 (5–7 WD40) repeats of RACK1 were identified as the minimal domain required for interaction with PIAS2 by deletion analysis. Furthermore, multiple PIAS2-domains, particularly the 'PINIT' and RLD domains, bind the RACK1 5–7 WD40 domain.

Structured summary of protein interactions:
PIAS2 physically interacts with **RACK1** by two hybrid (View interaction)
RACK1 and **PIAS2** colocalize by fluorescence microscopy (View interaction)
PIAS2 physically interacts with **RACK1** by two hybrid pooling approach (View interaction)
PIAS2 and **RACK1** colocalize by fluorescence microscopy (View interaction)
PIAS2 physically interacts with **RACK1** by anti bait coimmunoprecipitation (View Interaction: 1, 2)

1. Introduction

The human and mouse family of PIAS (protein inhibitor of activated STAT [signal transducer and activator of transcription]) proteins consists of PIAS1, PIASx (also called PIAS2), PIAS3, and PIASy proteins [1–7]. The human PIAS2 gene encodes two splice variants, PIASxα and PIASxβ [2–4]. The mouse PIAS2 gene has at least five isoforms (isoforms 1–5), which differ in the N- and C-terminii as a result of alternative splicing. PIAS proteins are characterized by an N-terminal SAP (scaffold attachment factor, acinus, and PIAS), a 'PINIT' motif, a C-terminal RLD (RING finger-like zinc binding domain) and an acidic domain [8–11]. PIAS proteins have SUMO E3-ligase activity and interaction of PIAS proteins with transcription factors often results in sumoylation of that protein [12–15]. In addition to sumoylation, PIAS proteins regulate gene expression by blocking the interaction of transcription factors with target DNA, by recruiting co-repressors and co-activators of transcription and by targeting proteins to nuclear bodies [16,17].

Although PIAS proteins were originally identified as proteins that bind to and inhibit STATs, the functions of PIAS proteins are clearly not limited to the regulation of STATs. PIAS proteins are capable of interacting with and modulating (activating and repressing) transcriptional activities of several transcription factors, including the androgen receptor (AR) [18–20].

In this study, RACK1 (receptor of activated C kinase 1) was identified as a PIAS2 partner through yeast two-hybrid screening of cDNA libraries derived from mouse spermatogonial stem cells and mouse stem cells using PIAS2 as bait. RACK1 is a homolog of the β-subunit of heterotrimeric G proteins, involved in membrane anchorage of multiple proteins including protein kinase C [21]. The detection of the PIAS2/RACK1 interaction in mammalian cells suggests that this interaction occurs in vivo. The minimal interacting regions of RACK1 and PIAS2 were identified by deletion analysis.

Abbreviations: STAT, signal transducer and activator of transcription; PIAS2, protein inhibitor of activated STATs 2; RACK1, receptor of activated protein kinase C 1; GNB2L1, guanine nucleotide-binding protein subunit 2-like 1; WD40, WD40 repeat domain; RLD, RING finger-like domain; SAP, Saf-A/B, acinus and Pias; SUMO, small ubiquitin-related modifier; AR, androgen receptor; X-α-Gal, 5-bromo-4-chloro-3-indolyl α-D-galactopyranoside; DMEM, Dulbecco's modification of Eagle's medium; SD, synthetic dropout medium; SD/-LTHA, synthetic complete medium lacking leucine, tryptophan, histidine and adenine; GFP, green fluorescent protein; FITC, fluorescein isothiocyanate; DAPI, 4′,6-diamidino-2-phenylindole; TRITC, tetrametrylrhodarnine isothiocyante

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0014-5793/$36.00
doi:10.1016/j.febslet.2011.12.013

2. Materials and methods

2.1. Yeast two-hybrid screening

The common cDNA fragment of the mouse PIAS2 gene (9–401aa corresponding to nucleotides (nt) 213–1391 of NM_008602) was cloned into the pGBKT7 vector containing the GAL4 DNA-binding domain to generate the bait plasmid, pGBKT7–PIAS2. This construct did not show toxic effects or autonomous transcriptional activation following transformation into the yeast strain, Y187. The mouse spermatogonial stem cell cDNA library and the mouse stem cell cDNA library were constructed in a pGADT7-Rec vector containing a GAL4 activation domain using Matchmaker Library Construction and Screening Kits (Clontech, Santa Clara, USA) and then transformed into the yeast AH109 strain. Yeast two-hybrid screening was performed using the MATCHMAKER two-hybrid system (Clontech). Positive clones were selected based on their ability to grow on synthetic dropout (SD) medium/-LTHA/X-α-Gal and for X-α-Gal activity.

2.2. Clone isolation and interaction analysis

Plasmid DNA of prey clones was isolated (Qiagen, Hilden, Germany) and transformed into *Escherichia coli* DH5α. Prey clones were recovered by ampicillin resistance and cDNA inserts were identified by PCR amplification, sequencing, and BLAST alignment. Interaction between the bait and identified prey clones was verified by co-transforming the purified prey plasmid with the bait pGBKT7–PIAS2 construct into the yeast AH109 strain followed by selection on SD/-LTHA medium. Co-transforming of pGBKT7-p53 with pGADT7-SV40 was used to be a positive control. Meanwhile, a negative control was also included by co-transforming of pGBKT7-p53 with pGADT7 vector into the same yeast cells.

2.3. Deletion mutant constructs of RACK1 and PIAS2 clones

Eight deletion mutant constructs of RACK1 163–326 in pGADT7 were constructed by sequential deletion of individual WD domains from the C- or N-terminii of the fragment. Amplified inserts were cloned into the pGEMT-Easy vector (Promega, Madison, USA) and subcloned into the pGADT7 vector. Correct identity and cloning orientation was confirmed by restriction digestion analysis and sequencing. Deletion mutant constructs were co-transformed with pGBKT7–PIAS2 into yeast strain AH109 and the resulting transformants were cultured on SD medium/-LTHA/X-α-Gal.

A panel of PIAS2 9–401aa deletion mutants were generated by PCR amplification of various portions of PIAS2 that were cloned into the pGBKT7 vector to create fusions with the C-terminal end of the GAL4 DNA-binding domain. All PCR products and mutagenized constructs were verified by sequencing before use. Deletion mutant constructs were co-transformed with pGADT7-RACK1 WD5–7 into yeast strain AH109 and the resulting transformants were cultured on SD medium/-LTHA/X-α-Gal.

2.4. Expression constructs and antibody preparation

The common cDNA fragment of the mouse PIAS2 gene was amplified by PCR and cloned into the pCMV-Myc and pDsRed -Express-1 vector (Clontech) to generate pCMV-c-Myc-PIAS2 and pDsRed-Express-1-PIAS2, respectively. Full-length RACK1 was cloned into pEGFP-N1 (Clontech) to generate pEGFP-RACK1.

To prepare PIAS2 polyclonal antibody, the common cDNA fragment of the PIAS2 gene was amplified and cloned into the pET28a expression vector (Novagen, Madison, WI) and used to transform Escherichia coli BL21 (DE3). The recombinant protein expression was induced with IPTG (isopropyl-1-thio-b-D-galactopyranoside) and the protein was purified with the Ni-activated His-binding resin (Yueda Company, Shanghai, China). The purified recombinant protein was refolded by graded urea series. Production of purified recombinant PIAS2 was used to immunize mice. The animals were killed ten days after the last boost, and titers above 1:10000 were observed in all animals by ELISA analysis. Then antibodies were used for immunoprecipitation and Western Blot analysis.

The following commercially available antibodies were used for immunoprecipitation and Western blot analysis: monoclonal anti-c-Myc antibody (Millipore Biotechnology, Bedford, MA, USA) and rabbit anti-RACK1 polyclonal antibody (Abcam, Cambridge, UK).

2.5. Cell culture and transfection

HeLa cells (human epithelial carcinoma line) were cultured and maintained in DMEM (Invitrogen) supplemented with 10% fetal bovine serum at 37 °C in a humidified atmosphere containing 5% CO_2. Transient transfection of HeLa cells (75–90% confluence) was performed using Lipofectamine 2000 (Invitrogen, Carlsbad, USA) according to the instructions provided by the manufacturer.

2.6. Co-immunoprecipitation and Western blot analysis

Since both PIAS2 and RACK1 were expressed in HeLa cells, the whole cell lysates were used to test for an association between endogenous PIAS2 and RACK1. In brief, the cells were washed with ice-cold PBS and harvested in 500 ml lysis buffer (150 mM NaCl, 50 mM Tris pH 8, 1% Nonidet P-40, 0.5% deoxycholate, and a protease inhibitor mixture [Roche Applied Science, Mannheim, Germany]). After incubation in lysis buffer for 60 min on ice, lysates were clarified by centrifugation for 20 min at 14000 rpm at 4 °C. Specific antibodies (8 μg) or its control IgG were added to the supernatant and incubated for 120 min at 4 °C prior to addition of 50 μl protein G-agarose followed by an overnight incubation at 4 °C. Samples were then centrifuged for 1 min in a microcentrifuge and washed with 1 ml lysis buffer, 1 ml washing buffer 2 (500 mM NaCl, 50 mM Tris pH 7.5, 0.1% Nonidet P-40, 0.05% deoxycholate) and 1 ml washing buffer (10 mM Tris pH 8, 0.1% Nonidet P-40, 0.05% deoxycholate). Initial lysates and immunoprecipitated proteins were analyzed by SDS–PAGE and immunoblotting was carried out using specific antibodies. Proteins were electrophor etically transferred to nitrocellulose membranes (Amersham Biosciences, Amersham, UK) and probed with the appropriate antibodies. Bound antibodies were visualized with horseradish peroxidase (HRP)-conjugated anti-rabbit or anti-mouse (Sigma) IgG antibodies using the enhanced chemiluminescence system (ECL, Thermo Scientific, MA, USA).

2.7. Immunofluorescence staining and imaging

To detect PIAS2 and RACK1, HeLa cells plated on round coverslips were transiently transfected with the pCMV-c-Myc-PIAS2 construct. After 24 h, cells were washed twice with PBS and fixed in 4% paraformaldehyde. Where indicated, cells were permeabilized with 0.1% Triton X-100 and further incubated for 1 h at room temperature with monoclonal mouse anti-c-Myc antibody and rabbit anti-RACK1 polyclonal antibody. Proteins were detected by incubation with TRITC-conjugated anti-mouse IgG and FITC-conjugated anti-rabbit IgG secondary antibodies for 90 min. Finally, coverslips were washed and mounted with Vectashield DAPI mounting medium (Vector Laboratories, Burlingame, CA, USA) and visualized by fluorescence microscopy.

3. Results and discussion

3.1. Identification of RACK1 as a PIAS2-interacting protein using a yeast two-hybrid screening system

To identify mouse proteins that interact with PIAS2, cDNA libraries derived from mouse spermatogonial stem cells and mouse stem cells were screened using a yeast two-hybrid system with the bait plasmid pGBKT7–PIAS2. Growth of yeast strain Y187 co-transformed with this construct and the empty prey plasmid pGADT7 was not supported on SD/-LTHA medium confirming that the bait was not auto-activated. A total of 5×10^6 primary transformants were screened, yielding over 150 positive clones, 58 of which demonstrated strong growth on SD/-LTHA medium. These clones were isolated, sequenced and aligned using the NCBI BLAST alignment search tool. Two independent clones encoded GNB2L1 (guanine nucleotide-binding protein subunit 2-like 1), also known as RACK1, spanning regions of 182 amino acids (RACK1 aa136–317) and 195 amino acids (RACK1 aa123–317), respectively, of the PIAS2 protein. To confirm these results, the PIAS2–RACK1 interaction was further investigated using a direct yeast hybrid screening system. Strong growth on SD/-LTHA medium was observed only in yeast cells co-transformed with pGBKT7–PIAS2 and pGADT7–RACK1 (Fig. 1), indicating interaction of RACK1 with PIAS2 in yeast.

3.2. RACK1 interacts with PIAS2 in vivo

RACK1 is an evolutionarily conserved protein belonging to the family of proteins that contain WD40 repeats, a domain involved in protein–protein interactions. Furthermore, RACK1 has been shown to interact with a wide range of proteins leading to the hypothesis that RACK1 acts as an adaptor protein [21–27]. The data obtained from the yeast two-hybrid screen was confirmed by evidence of a direct interaction between PIAS2 and RACK1 in mammalian cells. Co-immunoprecipitation and Western blot analyses were performed with proteins extracted from the human epithelial carcinoma HeLa cell line. RACK1 protein was immunoprecipitated from HeLa cells using a mouse polyclonal PIAS2 antibody prior to detection of RACK1 by Western blotting. And PIAS2 protein was also immunoprecipitated from HeLa cells using a rabbit RACK1 antibody prior to detection of PIAS2 by Western blotting. As shown in Fig. 2, a 35kD band or a 63Kd band corresponding to RACK1 and PIAS2 were observed in the co-immunoprecipitate and total lysate (input). These results indicated that RACK1 can interact with PIAS2 in mammalian cells.

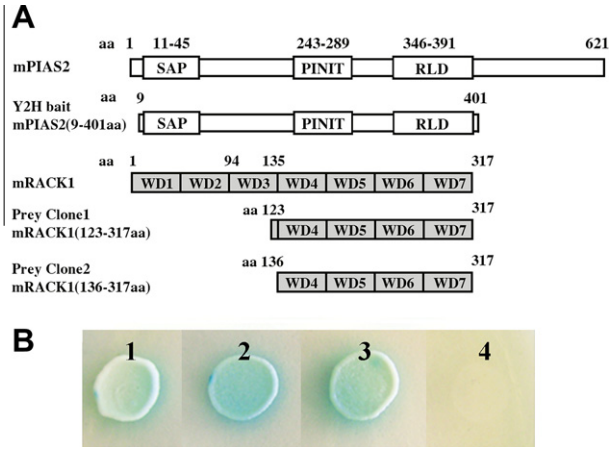

Fig. 1. Yeast two-hybrid bait and prey constructs and direct yeast two-hybrid analysis. (A) Bait construct. The common cDNA fragment of the mouse PIAS2 gene (9–401aa corresponding to nucleotides (nt) 213–1391 of NM_008602) was cloned into the pGBKT7 vector containing the GAL4 DNA-binding domain to generate the bait plasmid, pGBKT7–PIAS2. Two independent prey clones of RACK1 protein were identified. (B) Direct yeast two-hybrid analysis was used to confirm the interaction between RACK1 and PIAS2. Clone 1 is the positive control (pGBKT7-p53 and pGADT7-SV40 antigen). Clones 2 and 3 are RACK1 clone 1 and clone 2 co-transformed with the PIAS2 plasmid, respectively. Clone 4 is the negative control (pGBKT7-p53 and pGADT7).

3.3. Co-localization of RACK1 with PIAS2 in HeLa cells

Further confirmation of the interaction between RACK1 and PIAS2 proteins was obtained by immunostaining of pCMV-c-Myc-PIAS2 transfected HeLa cells. Two days after transfection, cells were stained with monoclonal mouse anti-c-Myc and rabbit anti-RACK1 polyclonal antibodies and protein expression was detected using TRITC-conjugated anti-mouse IgG and FITC-conjugated anti-rabbit IgG secondary antibodies, respectively. Cell nuclei were identified by DAPI (4,6-diamidino-2-phenylindole) staining. Staining was visualized by confocal laser microscopy (Fig. 3A). Microscopy showed that RACK1 (Fig. 3A, green) and PIAS2 (Fig. 3A, red) were co-localized in the whole cells (yellow) (Fig. 3A, MERGE).

The co-localiztion was also demonstrated by co-transfected pDsRed-Express-1-PIAS2 and pEGFP-RACK1 into Hela cells. As shown in Fig. 3B, GFP-RACK1 protein was uniformly distributed in the cells (Fig. 3B, green). And PIAS2 protein was scattered expressed in Hela cells (Fig. 3B, red). The merged image (Fig. 3B, yellow) revealed that RACK1 and PIAS2 were partially co-localized in Hela cells, indicating that RACK1 interacts with PIAS2 in Hela cells.

3.4. RACK1 5–7 WD40 repeats domain is essential for binding to PIAS2

The presence of WD40 repeats, which have been implicated in protein–protein interactions, within RACK1 suggested that one of these motifs may be responsible for the interaction with PIAS2 [28,29]. The two independent clones isolated using the yeast two-hybrid screening system, RACK1 123–317 aa and RACK1 136–317 aa, contained 3–7 and 4–7 WD40 repeats, respectively. The minimal domain of RACK1 required to interact with PIAS2 was investigated by deletion of residues from the N terminus of RACK1 123–317 aa or the C terminus of RACK1 1–317 aa. The 5′-truncated deletion mutants of RACK1 encoded RACK1 3–7, 4–7, 5–7, 6–7 and 7 WD40 repeats. The 3′-truncated deletion mutants of RACK1 encoded RACK1 1–4, 1–5 and 1–6 WD40 repeats. Deletion mutant constructs were analyzed for their ability to interact with PIAS2 in yeast. Resultant transformants were cultured on media lacking adenine, leucine, histidine and tryptophan selecting for both plasmids and for an interaction between the proteins encoded by these plasmids. Interaction between PIAS2 and RACK1 3–7, 4–7 and 5–7 deletion mutants was observed. No interaction was observed between the other RACK1 deletion mutants and PIAS2 (Fig. 4). These results indi-

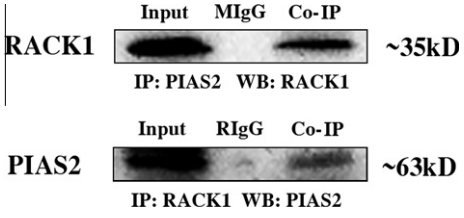

Fig. 2. PIAS2 co-immunoprecipitates with RACK1. HeLa cells were homogenized in lysis buffer. Then the cell lysates were incubated with anti-RACK1 antibody, Rabbit IgG (RIgG), anti-PIAS2 antibody and mouse IgG (MIgG) for immunoprecipitation (IP), respectively. Co-immunoprecipitants (Co-IP) were analyzed by Western Blot (WB) with anti-PIAS2 and anti-RACK1 antibodies, respectively. The total lysates (Input) were also analyzed.

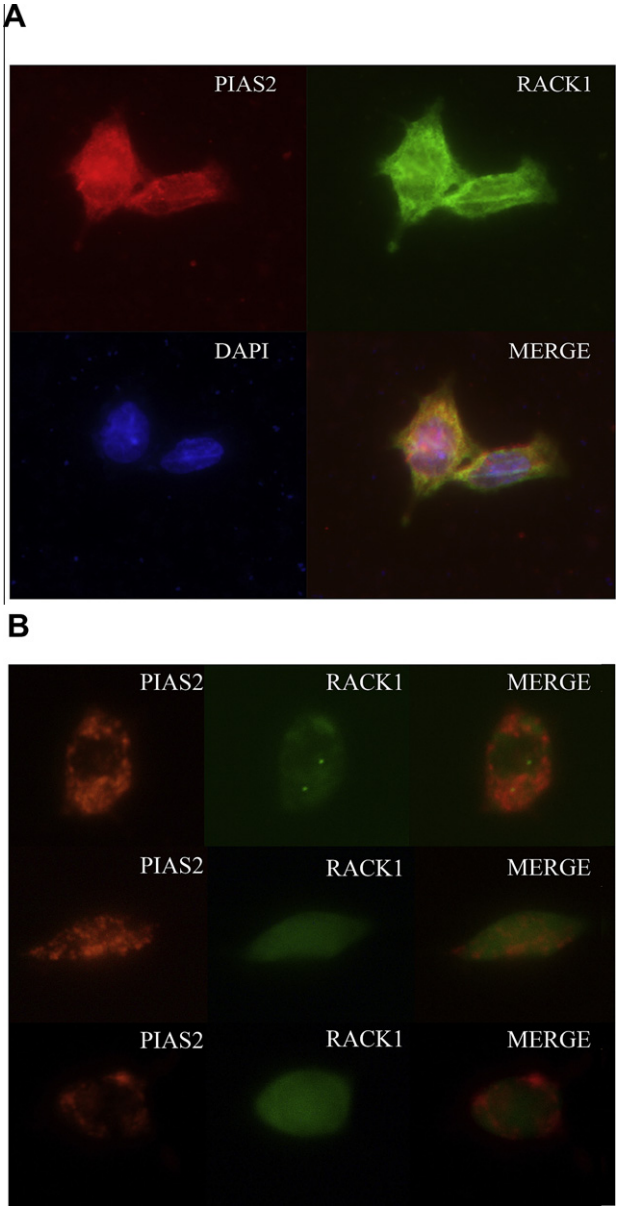

Fig. 3. Co-localization of RACK1 with PIAS2 in HeLa cells. (A) PIAS2 (red) and RACK1 (green) protein were visualized by immunostaining with monoclonal mouse anti-c-Myc and rabbit anti-RACK1 polyclonal antibodies and protein expression was detected using TRITC-conjugated anti-mouse IgG and FITC-conjugated anti-rabbit IgG secondary antibodies, respectively. Cell nuclei were stained by DAPI (blue). Staining was visualized by confocal laser microscopy. The merged image shows regions of signal overlap (yellow). (B) Hela cells were transfected with expression vectors for DsRed-PIAS2 and EGFP-RACK1 by Lipofectamine 2000. Two days after transfection, the cells were detected by fluorescence microscopy. The two figures [PIAS2 (red) and RACK1 (green)] have been merged.

cate that the RACK1 interaction domain of PIAS2 comprises at least 5–7 WD40 repeats.

3.5. Identification of the PIAS2-domain required for interaction with RACK1 5–7 WD40 repeats

In order to identify the PIAS2-domain required for interaction with RACK1 5–7 WD40 repeats, a series of N- and C-terminal truncated deletion mutants of PIAS2 9–401aa were generated and introduced in the pGBKT7 vector. These truncated mutants and pGADT7-RACK1 5–7 WD40 repeats were co-transformed into yeast

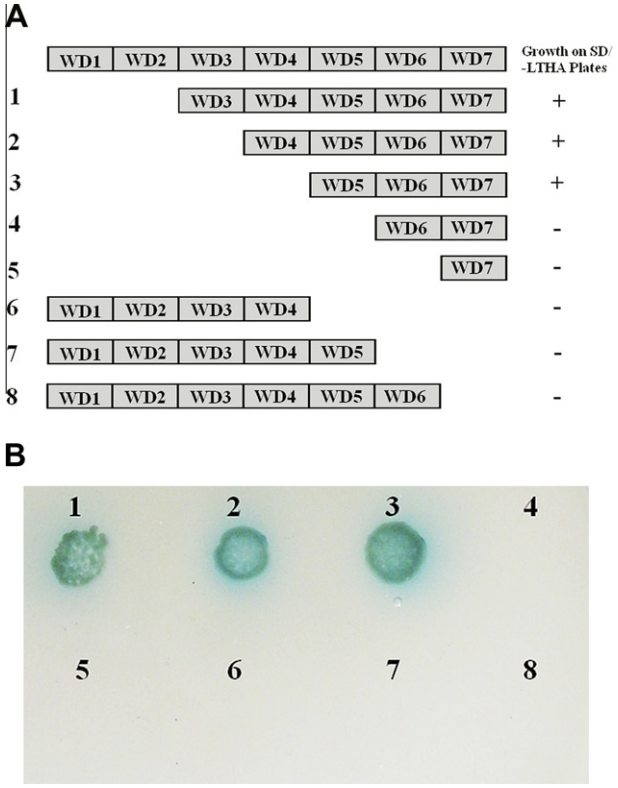

Fig. 4. WD40 repeats 5–7 of RACK1 are essential for binding to PIAS2. (A) Constructs encoding various regions of RACK1 were cloned into pGADT7 and analyzed for their ability to interact with PIAS2. The prey full length RACK1 protein including seven WD40 repeats is represented at the top. Constructs 1–8 represent truncated RACK1 deletion mutants. The interactions of truncated RACK1 deletion mutants with PIAS2 is represented by – and +. (B) Interaction of truncated RACK1 deletion mutants (1–8, as shown in A) with PIAS2 in yeast strain AH109.

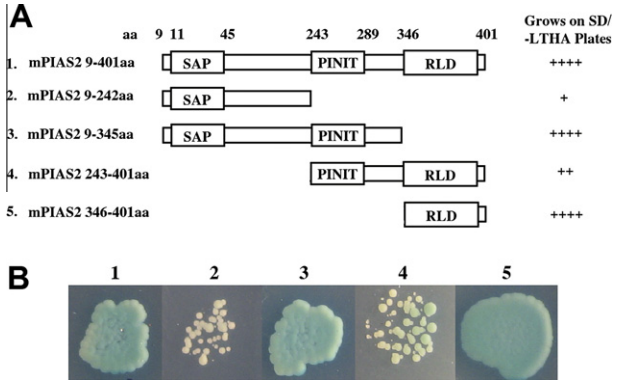

Fig. 5. Identification of the PIAS2-domain required for interaction with RACK1 5–7 WD40 domain. (A) Deletion analysis of the PIAS2-domains required for RACK1. The bait PIAS2 protein, including the location of known domains (1). PIAS2 amino acids expressed by each cDNA clone in pGBKT7 (1–5). Approximate relative yeast two-hybrid growth rates following co-transfection with the RACK1 5–7 WD40 prey plasmid are represented by ++++, ++, and +. Results suggested that multiple domains of the PIAS2 interacted with RACK1 5–7 WD40 domains, including the 'PINIT' and RLD domains. (B) Interaction of truncated PIAS2 clones with RACK1 5–7 WD40 domains in yeast strain AH109. Yeast containing the truncated bait PIAS2 (from 1–5, as indicated in A and B) and the prey RACK1 5–7 WD40 domain plasmids cultured on SD/-LTHA plates.

AH109 and the resulting transformants were cultured on selection medium (Fig. 5). Similar interaction was observed between RACK1 5–7 WD40 repeats and PIAS2 9–345aa, PIAS2 346–401aa and

PIAS2 9–401aa. PIAS2 9–242aa showed weak binding to RACK1 5–7 WD40 repeats and PIAS2 243–401aa binding was weaker still. It has been reported that PIAS2 protein contains several characterized domains. The SAP domain of PIAS2 (PIAS2 9–242aa) can bind to A/T-rich DNA and may involved in targeting PIAS proteins to nuclear scaffold [30]. The 'PINIT' motif is essential for nuclear retention of PIAS proteins [11]. So we supposed that the SAP domain may interact with RACK1 in nuclear of cells. When the 'PINIT' motif was disrupted from SAP domain, the protein may be exported from nuclear. And then the interaction will be inhibited. In contrast, the RLD domain (PIAS2 346–401aa) mediates the SUMO-E3-ligase activity of PIAS proteins and binds directly to Ubc9, the SUMO E2 enzyme. Deletion of the PINIT motif from RLD domain may facilitate this region releasing from nuclear and then interact with RACK1 in the cytoplasm of the cells. All in all, these results suggest that multiple PIAS2-domains, particularly the 'PINIT' and RLD domains, are involved in binding of RACK1 5–7 WD40 repeats.

In conclusion, this study identified RACK1 as a binding partner of PIAS2. Furthermore, WD40 repeats 5–7 of RACK1 were identified as the minimal domain required for interaction with PIAS2, while multiple domains of PIAS2, particularly the 'PINIT' and RLD domains, bind to the RACK1 protein.

Acknowledgments

This work was supported by grants from the National Natural Science Foundation of China (Nos. 31071020 and 81000272) and the Natural Science Foundation of Jiangsu Province of China (No. BK2009192).

References

[1] Liu, B., Liao, J., Rao, X., Kushner, S.A., Chung, C.D., Chang, D.D. and Shuai, K. (1998) Inhibition of Stat1-mediated gene activation by PIAS1. Proc. Natl. Acad. Sci. USA 95 (18), 10626–10631.
[2] Kotaja, N., Aittomäki, S., Silvennoinen, O., Palvimo, J.J. and Jänne, O.A. (2000) ARIP3 (androgen receptor-interacting protein 3) and other PIAS (protein inhibitor of activated STAT) proteins differ in their ability to modulate steroid receptor-dependent transcriptional activation. Mol. Endocrinol. 14 (20), 1986–2000.
[3] Fu, J., Xiong, Y., Xu, Y., Cheng, G. and Tang, H. (2010) MDA5 is SUMOylated by PIAS2β in the upregulation of type I interferon signaling. Mol. Immunol. 48 (4), 415–422.
[4] Arora, T., Liu, B., He, H., Kim, J., Murphy, T.L., Murphy, K.M., Modlin, R.L. and Shuai, K. (2003) PIASx is a transcriptional co-repressor of signal transducer and activator of transcription. J. Biol. Chem. 278 (24), 21327–21330.
[5] Zheng, Y., Zhou, Z.M., Yin, L.L., Li, J.M. and Sha, J.H. (2004) Molecular cloning and characterization of a novel splicing variant of PIASx. Acta Pharmacol. Sin. 25 (8), 1058–1064.
[6] Chung, C.D., Liao, J., Liu, B., Rao, X., Jay, P., Berta, P. and Shuai, K. (1997) Specific inhibition of Stat3 signal transduction by PIAS3. Science 278 (5344), 1803–1805.
[7] Zhang, C., Yuan, X., Yue, L., Fu, J., Luo, L. and Yin, Z. (2010) PIASy interacts with p73alpha and regulates cell cycle in HEK293 cells. Cell. Immunol. 263 (2), 235–240.
[8] Shuai, K. (2000) Modulation of STAT signaling by STAT-interacting proteins. Oncogene 19 (21), 2638–2644.
[9] Sharrocks, A.D. (2006) PIAS proteins and transcriptional regulation – more than just SUMO E3 ligases? Genes Dev. 20 (7), 754–758.
[10] Shuai, K. (2006) Regulation of cytokine signaling pathways by PIAS proteins. Cell Res. 16 (2), 196–202.
[11] Duval, D., Duval, G., Kedinger, C., Poch, O. and Boeuf, H. (2003) The 'PINIT' motif, of a newly identified conserved domain of the PIAS protein family, is essential for nuclear retention of PIAS3L. FEBS Lett. 554 (1–2), 111–118.
[12] Takahashi, Y., Iwase, M., Strunnikov, A.V. and Kikuchi, Y. (2008) Cytoplasmic sumoylation by PIAS-type Siz1-SUMO ligase. Cell Cycle 7 (12), 1738–1744.
[13] Palvimo, J.J. (2007) PIAS proteins as regulators of small ubiquitin-related modifier (SUMO) modifications and transcriptional. Biochem. Soc. Trans. 35 (Pt6), 1405–1408.
[14] Rytinki, M.M., Kaikkonen, S., Pehkonen, P., Jääskeläinen, T. and Palvimo, J.J. (2009) PIAS proteins, pleiotropic interactors associated with SUMO. Cell. Mol. Life Sci. 66 (18), 3029–3041.
[15] Jackson, P.K. (2001) A new RING for SUM wrestling transcriptional responses into nuclear bodies with PIAS family E3 SUMO ligases. Genes Dev. 15 (23), 3053–3058.
[16] Tan, J.A., Hall, S.H., Hamil, K.G., Grossman, G., Petrusz, P. and French, F.S. (2002) Protein inhibitors of activated STAT resemble scaffold attachment factors and function as interacting nuclear receptor coregulators. J. Biol. Chem. 277 (19), 16993–17001.
[17] Schmidt, D. and Müller, S. (2003) PIAS/SUM new partners in transcriptional regulation. Cell. Mol. Life Sci. 60 (12), 2561–2574.
[18] Peng, Y., Lee, J., Zhu, C. and Sun, Z. (2010) A novel role for protein inhibitor of activated STAT (PIAS) proteins in modulating the activity of Zimp7, a novel PIAS-like protein, in androgen receptor-mediated transcription. J. Biol. Chem. 285 (15), 11465–11475.
[19] Gross, M., Liu, B., Tan, J., French, F.S., Carey, M. and Shuai, K. (2001) Distinct effects of PIAS proteins on androgen-mediated gene activation in prostate cancer cells. Oncogene 20 (29), 3880–3887.
[20] Takahashi, K., Taira, T., Niki, T., Seino, C., Iguchi-Ariga, S.M. and Ariga, H. (2001) DJ-1 positively regulates the androgen receptor by impairing the binding of PIASxα to the receptor. J. Biol. Chem. 276 (40), 37556–37563.
[21] Besson, A., Wilson, T.L. and Yong, V.W. (2002) The anchoring protein RACKI links protein kinase cepsilon to integrin beta chains, requirements for adhesion and motility. J. Biol. Chem. 277 (24), 22073–22084.
[22] Hermanto, U., Zong, C.S., Li, W. and Wang, L.H. (2002) RACK1, an insulin-like growth factor I (IGF-I) receptor-interacting protein, modulates IGF-I-dependent integrin signaling and promotes cell spreading and contact with extracellular matrix. Mol. Cell. Biol. 22 (7), 2345–2365.
[23] Kiely, P.A., Sant, A. and O'Connor, R. (2002) RACKI is an insulin-like growth factor I (IGF-I) receptor-interacting protein that can regulate IGF-I-mediated Akt activation and protection from cell death. Biol. Chem. 277 (25), 22581–22589.
[24] Kraus, S., Gioeli, D., Vomastek, T., Gordon, V. and Weber, M.J. (2006) Receptor for activated C kinase 1 (RACK1) and Src regulate the tyrosine phosphorylation and function of the androgen receptor. Cancer Res. 66 (22), 11047–11054.
[25] Wehner, P., Shnitsar, I., Urlaub, H. and Borchers, A. (2011) RACK1 is a novel interaction partner of PTK7 that is required for neural tube closure. Development 138 (7), 1321–1327.
[26] Ikebuchi, Y., Takada, T., Ito, K., Yoshikado, T., Anzai, N., Kanai, Y. and Suzuki, H. (2009) Receptor for activated C-kinase 1 regulates the cellular localization and function of ABCB4. Hepatol. Res. 39 (11), 1091–1107.
[27] He, D.Y., Neasta, J. and Ron, D. (2010) Epigenetic regulation of BDNF expression via the scaffolding protein RACK1. J. Biol. Chem. 285 (25), 19043–19050.
[28] Bird, R.J., Baillie, G.S. and Yarwood, S.J. (2010) Interaction with receptor for activated C-kinase 1 (RACK1) sensitizes the phosphodiesterase PDE4D5 towards hydrolysis of cAMP and activation by protein kinase C. Biochem. J. 432 (1), 207–216.
[29] Steele, M.R., McCahill, A., Thompson, D.S., MacKenzie, C., Isaacs, N.W., Houslay, M.D. and Bolger, G.B. (2001) Identification of a surface on the beta-propeller protein RACK1 that interacts with the cAMP-specific phosphodiesterase PDE4D5. Cell. Signal. 13 (7), 507–513.
[30] Okubo, S., Hara, F., Tsuchida, Y., Shimotakahara, S., Suzuki, S., Hatanaka, H., Yokoyama, S., Tanaka, H., Yasuda, H. and Shindo, H. (2004) NMR structure of the N-terminal domain of SUMO ligase PIAS1 and its interaction with tumor suppressor p53 and A/T-rich DNA oligomers. J. Biol. Chem. 279 (30), 31455–31461.